

Advantage to lower body temperatures for a small mammal (*Rattus fuscipes*) experiencing chronic cold

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Cold environments are challenging for small endotherms because they must increase heat production to compensate for increased heat loss to the environment. Mammals that endure cold without entering torpor may have thicker fur and a lower body temperature (T_b) than those in warm environments to save energy. Activity levels also may be reduced in the cold. We aimed to determine whether activity level, exposure to chronic cold, or an interaction between the 2 could reduce the T_b of an endotherm. Wild bush rats (*Rattus fuscipes*) were brought into captivity and acclimated to cold (12°C) or warm (22°C) ambient temperatures and were either sedentary or exercised. Daily exercise increased maximum T_b in cold-acclimated bush rats but also decreased minimum T_b . Mean ($\pm SE$) daily T_b was significantly lower in cold-acclimated bush rats (sedentary: $36.5^\circ\text{C} \pm 0.1^\circ\text{C}$; exercised: $36.4^\circ\text{C} \pm 0.2^\circ\text{C}$) compared to warm-acclimated bush rats (sedentary: $37.3^\circ\text{C} \pm 0.2^\circ\text{C}$; exercised: $37.0^\circ\text{C} \pm 0.1^\circ\text{C}$). Body temperature decreased significantly over the time of cold acclimation. Thicker fur and lower T_b s in cold-acclimated bush rats conferred an energy saving equivalent to 11% of daily food intake in exercised rats and 22% of daily food intake in sedentary rats. The lower T_b s recorded in some small mammals in winter are not necessarily due to lower levels of activity but can result from a regulated reduction in T_b in response to cooler ambient temperatures. DOI: 10.1644/10-MAMM-A-003.1.

Key words: body temperature, bush rat, exercise, fur thickness, heat generation model, *Rattus fuscipes*, thermal acclimation

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Endothermy affords benefits such as high aerobic capacity and extended periods of activity (Bennett and Ruben 1979; Portner 2004), but it is energetically expensive. Small endotherms especially are challenged by life in the cold (Humphries 2005), particularly if they remain active during winter rather than using torpor as an energy-conservation strategy. Some small endotherms have a lower body temperature (T_b) and thicker fur in winter, reducing the ingested energy required to maintain T_b compared to summer (Brown and Downs 2006; Glanville and Seebacher 2010; Pereira et al. 2002). Body temperature also correlates positively with activity levels (Pereira et al. 2002), and activity levels may be reduced in winter (Churchfield 1982; Scantlebury et al. 2005). Lower T_b s therefore could be a symptom of low activity levels rather than a regulated reduction in T_b .

In cold environments heat loss is increased, and endotherms need to increase heat production (Schmidt-Nielsen 1997; Scholander et al. 1950a). Therefore, when exposed to low environmental temperatures, mammals increase adaptive thermogenesis (Heldmaier 1989; Klingenspor 2003; Lowell 1998; Lowell and Spiegelman 2000). Some also increase insulation to reduce heat loss (Lovegrove 2005; Scholander et

al. 1950b). To reduce energy expenditure in response to low ambient temperatures (T_a), many mammals reduce metabolic rate and T_b and enter torpor or hibernation (Christian and Geiser 2007; Geiser 2004; Heldmaier 1989). Endotherms that do not use torpor can increase energy savings above those afforded by increased insulation by reducing T_b slightly in cold T_a s, for example, in winter (Glanville and Seebacher 2010). A lower T_b reduces the difference between T_b and T_a , and decreases requirements for metabolic heat production (Brown and Downs 2006; Pereira et al. 2002). The reduction in T_b is less substantial than that that accompanies torpor, and unlike torpid animals, activity is maintained.

Reducing T_b in the absence of any decrease in activity levels is complicated by the effect of physical activity on T_b (Thoden et al. 1994). Approximately 80% of the energy released when ATP is hydrolyzed to power muscle contractions during exercise is lost as heat (Reilly and Waterhouse 2009), so that T_b increases during exercise (Brooks et al. 1971; Kenny et al. 1997; Thoden et al. 1994). The lower T_b s recorded in some wild



mammals in winter therefore may be caused by decreased activity levels. Lower activity levels decrease exposure to low T_{as} for those animals that seek shelter in nests or burrows. However, a lower activity level also reduces foraging time. These animals may become more gregarious during winter to ensure that sufficient food is gathered despite reduced activity levels and thus foraging time (Pereira et al. 2002). If activity does directly influence T_b , the T_b of sedentary individuals should be lower than that of active individuals.

We aimed to determine the interactive effect of cold exposure and activity on the T_b of a small endotherm, the bush rat (*Rattus fuscipes*). We tested the hypotheses that the T_b of sedentary bush rats will be significantly lower than that of exercised bush rats and that T_b will be further reduced in cold-acclimated sedentary individuals, but daily exercise will elevate T_b above that of sedentary bush rats and lessen any cold-induced reduction in T_b . Bush rats are small (80–150 g) native Australian rodents that do not enter torpor and are active throughout the year. Body temperature in wild bush rats is significantly lower in winter than in summer (Glanville and Seebacher 2010). It is not known whether this reduction is due to lower T_{as} , or changes in photoperiod, food availability, or activity levels associated with winter, all of which could influence T_b (Deveci and Egginton 2002; Li and Wang 2007; Pereira et al. 2002; Wang et al. 2006). We tested our hypotheses with a thermal acclimation treatment and an exercise treatment, allowing us to test directly whether T_b is reduced by an interaction between reduced activity and chronic low T_{as} or by low T_{as} alone.

MATERIALS AND METHODS

Animal husbandry and body temperature.—Bush rats (mass range 80- to 150-g, median 115 g) were trapped in Elliott traps (9 × 10 × 33 cm; Elliott Scientific Equipment, Upwey, Victoria, Australia) baited with peanut butter, oats, and honey in Ku-ring-gai and Garigal National Parks, New South Wales, Australia (33°43'S, 151°14'E). All rats were checked for reproductive status, and obviously pregnant or lactating animals were released. Rats were transported to the University of Sydney and kept individually in plastic cages (240 × 300 × 340 mm) with newspaper bedding, polyvinyl chloride piping for shelter, branches for enrichment, and ad libitum food and water. Photoperiod was set at 12L:12D (0600 and 1800 h). Body temperature was continuously recorded at 30-min intervals by temperature data loggers ($\pm 0.5^\circ\text{C}$ and mass = 3 g; iButton thermochron; Dallas Semiconductor, Maxim Integrated Products Inc., Sunnyvale, California) implanted into the peritoneal cavity via ventral midline incisions. Transmitters weighed <5% of body mass of the smallest individual and were calibrated before implantation at 2°C intervals between 30°C and 40°C in a water bath, which was calibrated with a certified thermometer. Surgery was performed in sterile conditions under a general anesthetic (4% isoflurane for induction and 2% isoflurane for maintenance of anesthesia). The incision was closed with 4-0 PDS sutures at the muscle and skin layers, and surgical glue was applied over

the skin layer sutures to prevent disruption of the sutures by the bush rats. Bush rats were euthanized at the completion of acclimation treatments by an overdose of sodium pentobarbitone (120 mg/kg) injected into the peritoneal cavity, and the data loggers were recovered. Mean, maximum, and minimum daily T_b over the final week of acclimation were calculated for each rat, and daily T_b amplitude was calculated as the difference between the daily maximum and minimum T_b s. Sample sizes were $n = 10$ (5 males and 5 females) per acclimation treatment in the sedentary treatment and $n = 8$ (4 males and 4 females) per acclimation treatment in the exercise treatment. However, the failure rate of data loggers was 20% in the warm sedentary rats, reducing the sample size to 8 (4 males and 4 females). Failure rate was 37% in the exercised rats, reducing the sample size for T_b to 5 in both cold exercised (3 males and 2 females) and warm exercised rats (2 males and 3 females). We suspect battery failure caused the data logger failures. All experimental procedures were approved by the University of Sydney Animal Ethics Committee (permit L04/12-2006/3/4512) and the New South Wales National Parks and Wildlife Services (scientific license S12186) and adhered to the guidelines established by the American Society of Mammalogists (Gannon et al. 2007).

Acclimation and exercise treatments.—Two weeks after surgery bush rats were allocated randomly to either a cold (12°C) or warm (22°C) acclimation treatment. Acclimation temperatures were selected based on mean minimum summer and winter air temperatures for coastal Sydney, Australia. The thermal neutral zone of bush rats is 29–32°C (Collins 1973; E. J. Glanville, pers. obs.), which the rats from the mideast coast of New South Wales, Australia, almost never experience in the wild due to their nocturnal habits (Glanville and Seebacher 2010). Therefore the acclimation temperatures are 7°C and 17°C below the lower bounds of the thermal neutral zone. Room temperature was monitored using precalibrated temperature data loggers (iButton thermochron; Dallas Semiconductor). The temperature of the acclimation rooms was decreased gradually from 22°C to 12°C by 2°C/day over 1 week prior to the commencement of acclimation and the start of recording by the iButtons. Within each thermal acclimation group rats were allocated to either a sedentary or an exercise treatment, resulting in a warm-acclimated sedentary (WS), warm-acclimated exercised (WE), cold-acclimated sedentary (CS), and cold-acclimated exercised (CE) treatment. Throughout the duration of the 30-day acclimation period rats in the exercise treatment were removed from their cages on 5 of every 7 days and placed individually in an enclosed, motorized running wheel. Rats were run for 30 min at 60% of the speed previously determined in ramped-speed running trials to result in a plateau in oxygen consumption indicating maximum exercise-induced metabolic rate (E. J. Glanville, pers. obs.). The duration and frequency of exercise was identical between thermal acclimation treatments. Feeding and photoperiod regimes were identical in all treatments. Handling regimes were identical between cold and warm sedentary treatments.

At the end of the acclimation treatments all rats were placed in temperature-controlled rooms at 12°C or 22°C on consecutive days for a minimum of 4 h to test whether any difference in T_b was maintained regardless of T_a (Yang and Gordon 1996). Mean T_b over the final hour of exposure was used to determine statistically whether any treatment-induced differences in T_b were maintained.

Food intake.—Apparent food consumption, calculated as the difference between food given and food remaining (Rezende et al. 2009), was considered an estimate of daily food intake. Intake was measured over five 24-h periods at the end of acclimation ($n = 5$ sedentary and $n = 8$ exercised, per thermal acclimation treatment). During this time, dry food (25 g rodent mix; energy content 24.5 kJ/g) and diced apple (15 g; energy content 2.1 kJ/g) were preweighed using an electronic balance (± 0.01 g; Sartorius, Goettingen, Germany) and offered in a food bowl on a plastic tray to ensure all remaining food could be weighed correctly. All rats were weighed before, at the midpoint of, and after acclimation treatments on an electronic balance (correct to ± 0.01 g; Sartorius). To determine how treatments affected body mass, a ratio of postacclimation to preacclimation weight was calculated for each individual.

Fur thickness.—A sample of 20 guard (long, thick, straight, black) and 20 undercoat (short, fine, crimped) hairs were selected from within 1 cm² in an identical position on the middorsal region of the coat of all rats. Effective fur thickness was estimated according to Reynolds (1993). Hair diameter (δ ; cm) was measured to the nearest 0.001 mm using a precalibrated micrometer. Length was measured to the nearest 0.01 mm at 10 \times magnification (L_f ; cm). Within a precisely measured 1-cm² shaved square of skin, density of fur (hairs/cm²) was estimated by counting hair stubs within 10 randomly selected 1-mm² squares under 10 \times magnification. The average hair stubs per square millimeter was determined and multiplied by 100 to give an estimation of hair density per square centimeter (ρ ; hairs/cm²). The average angle of hair to the skin surface (θ) when piloerect (0°) and when lying naturally against the skin (45°) also was determined. Individual hairs were assumed to resemble a cylinder, and hence an index of effective thickness of fur per unit volume of fur (τ) was calculated according to the formula $\tau = \rho L_f (\pi \delta^2 / 4) (\cos \theta)$ (Reynolds 1993). Absolute fur thickness of each rat was determined empirically by measuring fur thickness with vernier calipers by taking the distance from the skin surface to the light extinction mark at the top of the fur layer (Reynolds 1993).

Statistical analysis.—We present T_b over the time course of acclimation as daily means and mean daily maxima and minima and amplitude for each treatment group at 5-day intervals, from day 1 to day 30. Two-way repeated-measures analyses of variance (ANOVAs) were used to analyze the change of mean, maximum, minimum, and amplitude of T_b during the treatments and also were used to determine whether any differences in T_b were maintained when bush rats were subjected to different T_a s. One-way ANOVA was used to determine significant differences in mean T_b , body mass

ratios, fur thickness, and daily food intake between treatment groups. We performed Tukey's post hoc tests when ANOVAs were significant. Statistical tests were performed in SPSS 16.0 (SPSS Inc., Chicago, Illinois). The level of significance was set at $\alpha = 0.05$. Sample size was $n = 10$ for CS and WS bush rats and $n = 8$ for CE and WE bush rats. Unless stated otherwise, data are expressed as mean \pm standard error (*SE*).

RESULTS

Body temperature.—Body temperature was lower in cold-acclimated (Fig. 1B) compared to warm-acclimated (Fig. 1A) bush rats, irrespective of whether bush rats were exercised or sedentary. Mean daily T_b was significantly lower in the cold-acclimated bush rats than in the warm-acclimated bush rats (Table 1). Mean daily minima were significantly lower in the CE bush rats than in the CS bush rats, and this was significantly lower than in the warm-acclimated bush rats. Mean daily maxima were lower in the CS bush rats than in all other treatment groups. Body temperature amplitude was significantly greater in the CE bush rats compared to all other bush rats (Table 1). The T_b of cold-acclimated bush rats was significantly lower than that of warm-acclimated bush rats at both 12°C and 22°C, regardless of whether the rats were exercised or sedentary ($F_{3,14} = 13.97$, $P < 0.01$; Fig. 1C). We observed no significant effect of T_a on T_b ($F_{1,14} = 2.46$, $P = 0.14$), and no significant interaction occurred between treatment and T_a ($F_{3,14} = 1.72$, $P = 0.21$).

Mean daily T_b decreased over the time of cold acclimation regardless of exercise treatment, with a significant interaction between time and treatment ($F_{18,144} = 2.37$, $P < 0.01$; Fig. 2A). There was a significant interaction in daily maximum T_b between time and treatment ($F_{18,144} = 1.77$, $P < 0.05$; Fig. 2B). A significant interaction was found between time and treatment for minimum T_b ($F_{18,144} = 2.13$, $P < 0.001$; Fig. 2C) and minimum T_b decreased over the time course of acclimation in CE bush rats. Body temperature amplitude increased over the time course of acclimation in exercised bush rats with a significant interaction between treatment and time ($F_{18,144} = 2.49$, $P < 0.01$; Fig. 2D).

Mass, fur thickness, and food intake.—Neither thermal acclimation nor exercise training had a significant effect on body mass, with no significant difference in the ratio of body mass postacclimation to body mass preacclimation (CE: 0.97 ± 0.11 ; CS: 1.01 ± 0.04 ; WE: 0.94 ± 0.03 ; WS: 1.01 ± 0.02 ; $F_{3,25} = 3.05$, $P = 0.90$). Effective fur thickness was significantly different among treatments ($F_{3,35} = 55.27$, $P < 0.001$; Fig. 3). Effective fur thickness did not differ between WE and WS bush rats but was significantly thicker in CE bush rats and was thickest in CS bush rats. Absolute fur thickness followed the same pattern, with the fur of CS bush rats being significantly thicker than that of CE bush rats, which was significantly thicker than fur from all warm-acclimated bush rats (Table 1). Total daily food intake (kJ/day) did not differ between CS and WS bush rats but was significantly higher in WE bush rats and highest in CE bush rats (Table 1).

DISCUSSION

The T_b of bush rats decreased by up to 0.9°C in response to chronic exposure to cold and in the absence of any change in photoperiod. The T_b of cold-acclimated bush rats decreased over the first 10–14 days of cold acclimation before stabilizing. Fur thickness also increases with cold acclimation, more so in sedentary bush rats than in exercised bush rats. After acclimation, CE bush rats had significantly lower minimum $T_{b,s}$ than CS bush rats. Therefore, a decrease in activity is unlikely to explain the lower T_b observed in winter in wild bush rats (Glanville and Seebacher 2010). Heat generation calculations demonstrate that the cold-acclimation-induced decrease in T_b contributes to a strategy that reduces the amount of energy required by small mammals in cold environments.

Cold acclimation induces a similar reduction in T_b in captive bush rats as winter induces in wild bush rats (Glanville and Seebacher 2010). Lower $T_{b,s}$ in winter also have been reported in some other wild mammals (Brown and Downs 2006; Fuglestad et al. 2006; Merritt et al. 2001; Pereira et al. 2002). Wild animals are faced with an array of environmental cues that could affect T_b and that occur simultaneously with decreased T_a (Haim and Rubal 1993; Lovegrove 2005; Pereira et al. 2002). We have shown that T_a alone can initiate a change in T_b . A slight decrease in T_b occurs immediately following commencement of cold-acclimation. The cold-induced decrease in T_b continues over 2 weeks of chronic exposure to 12°C. Therefore, readjustment of T_b requires a repeated thermal cue from the external environment. The progressive reduction in environmental temperature that signals the onset of winter would provide this cue for animals in the wild.

The lower T_b of cold-acclimated bush rats persisted even when they were placed in warmer temperatures for several hours. Similarly, warm-acclimated bush rats maintained their higher $T_{b,s}$ when placed in cooler environments. Thus, the lower T_b of cold-acclimated bush rats may be a new T_b balance point resulting from a change in the temperature threshold for the activation of thermoeffectors (Romanovsky 2007) rather than an acute response to the thermal environment. The absence of any difference in photoperiod between treatments removes the effect of the pineal gland and its role in regulating T_b via the photosensitive release of melatonin (Cassone et al. 1993). The decrease in T_b in response to low $T_{a,s}$ supports the importance of sensors of the thermal

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FIG. 1.—Mean body temperature (T_b) of bush rats at 30-min intervals over 48 h at the conclusion of a 30-day A) warm-acclimation (22°C) treatment (sedentary: $n = 8$; exercised: $n = 5$) and B) cold-acclimation (12°C) treatment (sedentary: $n = 10$; exercised: $n = 5$). Bush rats are nocturnal, and peaks in T_b coincide with the dark phase. Spikes in maximum T_b in the exercised treatments coincide with exercise bouts. All SEs are $<0.1^\circ\text{C}$. C) Body temperature of cold-acclimated and warm-acclimated sedentary and exercised bush rats when subjected to either 12°C or 22°C for 4 h. Different letters indicate a significant difference ($P < 0.05$) between treatments at each test temperature.

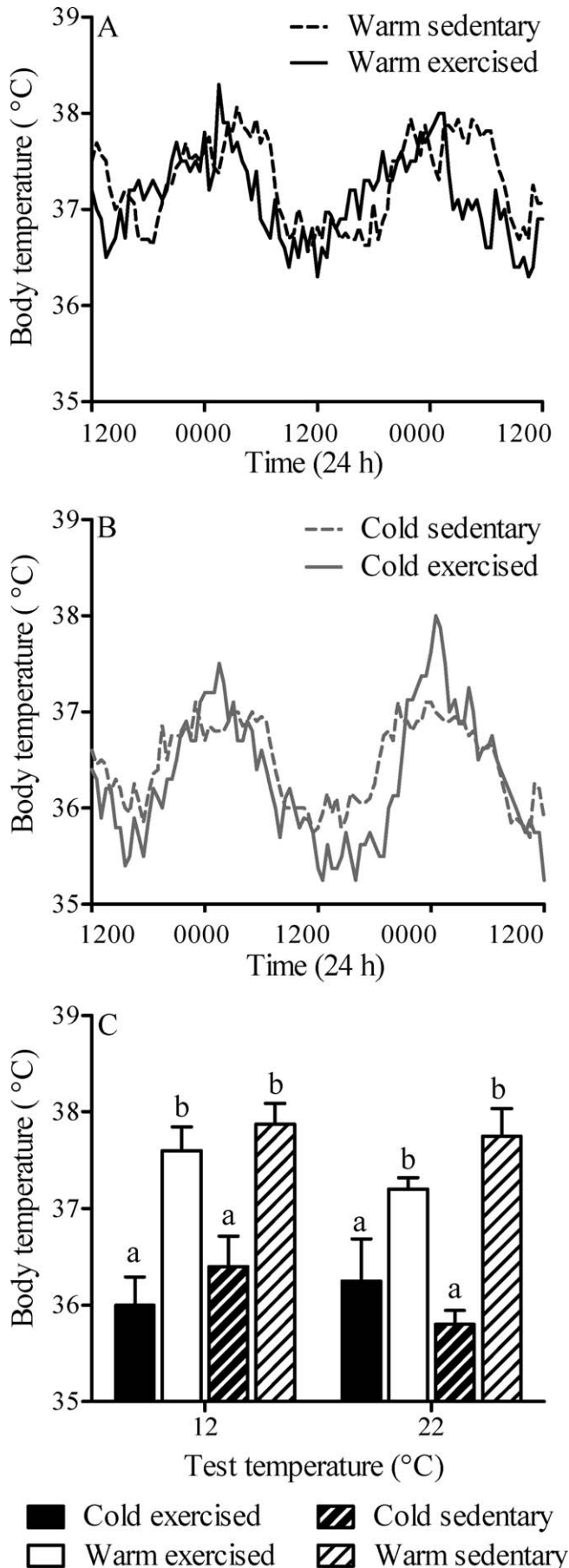


TABLE 1.—Mean ($\pm SE$) body temperature (T_b ; $^{\circ}C$), empirically derived fur thickness (mm), and daily food intake (kJ/day) of cold-acclimated ($12^{\circ}C$) exercised (CE) and sedentary (CS) and warm-acclimated ($22^{\circ}C$) exercised (WE) and sedentary (WS) bush rats. Daily T_b parameters were determined in the last week of a 30-day acclimation period. Different superscript letters indicate significant differences ($P < 0.05$) between treatments within a parameter as determined by post hoc analysis.

Parameter	Treatment				F	d.f.	P
	CE	CS	WE	WS			
Mean T_b	36.4 \pm 0.2 ^a	36.5 \pm 0.1 ^a	37.0 \pm 0.1 ^b	37.3 \pm 0.2 ^b	8.98	3,27	<0.0001
Maximum T_b	38.4 \pm 0.3 ^a	37.8 \pm 0.1 ^b	38.5 \pm 0.3 ^a	38.6 \pm 0.2 ^a	3.84	3,27	<0.05
Minimum T_b	34.9 \pm 0.2 ^a	35.4 \pm 0.2 ^b	35.7 \pm 0.2 ^b	36.1 \pm 0.2 ^c	9.69	3,27	<0.001
T_b amplitude	3.5 \pm 0.1 ^a	2.4 \pm 0.1 ^b	2.8 \pm 0.3 ^b	2.4 \pm 0.2 ^b	7.31	3,27	<0.05
Fur thickness	13.2 \pm 0.00 ^a	13.6 \pm 0.00 ^b	8.6 \pm 0.00 ^c	8.6 \pm 0.01 ^c	586.04	3,32	<0.001
Food intake	145.6 \pm 14.53 ^a	80.2 \pm 8.37 ^c	98.5 \pm 9.22 ^b	65.2 \pm 8.05 ^c	9.30	3,25	<0.001

environment in determining the point to which T_b is regulated. Thermosensation is facilitated by transient receptor potential ion channels, which are distributed throughout the body and in the skin (Caterina 2007; Patapoutian et al. 2003). Temperature activates these channels, each of which is gated by a characteristic temperature range (Caterina 2007; McKemy 2007; Patapoutian et al. 2003). The point to which rodents regulate T_b and their behavioral responses to the thermal environment can be altered by blocking or knocking out

specific transient receptor potential ion channels (Colburn et al. 2007; Dhaka et al. 2007; Gava 2008; Kanizsai et al. 2009; Mills et al. 2008). Similar mechanisms may occur in bush rats. Hence, chronic exposure to a lower T_a appears to alter the threshold for the activation of thermoeffectors resulting in cold-acclimated bush rats maintaining their lower T_b despite experiencing a warm environment.

Minimum daily T_b was lowest in CE bush rats. This was surprising because we expected that sedentary animals would

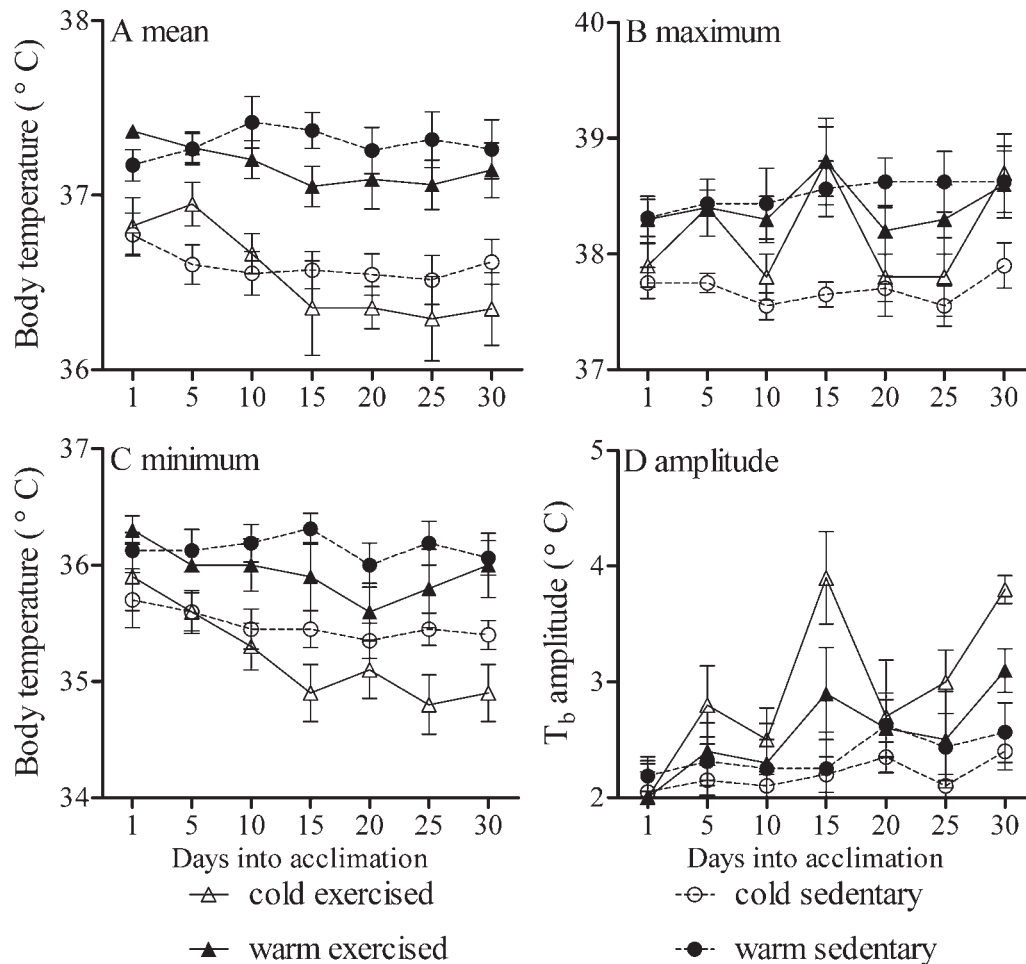


FIG. 2.—Mean ($\pm SE$) body temperatures (T_b s) over the duration of acclimation in cold-acclimated sedentary ($n = 10$) and exercised ($n = 5$) bush rats and warm-acclimated sedentary ($n = 8$) and exercised ($n = 5$) bush rats. A) Mean daily T_b ; B) maximum daily T_b ; C) minimum daily T_b ; D) body temperature amplitude.

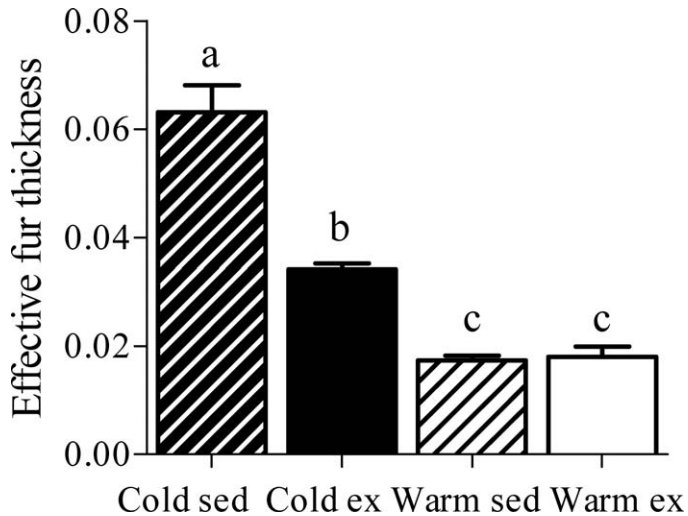


FIG. 3.—Mean (\pm SE) index of effective fur thickness (see “Materials and Methods” for derivation) in cold-acclimated sedentary (Cold sed) and exercised (Cold ex) bush rats and warm-acclimated sedentary (Warm sed) and exercised (Warm ex) bush rats. Different letters indicate significant differences ($P < 0.05$).

have lower T_b s than active animals because activity correlates with T_b (Bolles et al. 1968; Kluger et al. 1973; Refinetti and Menaker 1992). A short bout of daily exercise did have the expected effect of increasing maximum T_b (Brooks et al. 1971). However, this occurred only in cold-acclimated rats. Exercise did not increase daily maxima in warm-acclimated bush rats. The effect of an exercise-induced increase in maximum T_b on mean daily T_b was balanced by a significantly lower minimum T_b resulting in increased daily amplitude in CE bush rats. This might be explained by the greater demand for ATP and heat production placed on animals facing the combined energetic challenges of locomotion and thermoregulation in a cold environment. Daily T_b amplitude increased on days when exercise took place, demonstrating a direct effect of exercise on T_b . Thermal acclimation alone had no effect on T_b amplitude.

The T_b amplitude of the acclimated bush rats was relatively high compared with other *Rattus* species (Refinetti and Menaker 1992). This appears to be due to the lower minimum T_b s experienced by bush rats. However, the T_b amplitude of warm-acclimated bush rats was median when compared to T_b amplitude of a selection of similar-sized mammals maintained at 24°C (Refinetti 1999). The T_b amplitude of captive bush rats was lower than it was in wild bush rats (Glanville and Seebacher 2010). The effect of exercise on T_b amplitude suggests that the difference between wild and captive bush rats is due to longer and more strenuous periods of activity exhibited by wild rats. Additionally, the difference in mean T_b between summer- and winter-acclimatized rats (0.4°C—Glanville and Seebacher 2010) was less than the difference between cold- and warm-acclimated rats (up to 0.9°C). The constant T_a in captivity, compared to the variable environmental temperatures in the wild, could explain this difference because the thermal cue is more consistent and therefore stronger in captivity.

The thermal neutral zone of *Rattus fuscipes* is 29–32°C and does not change with cold acclimation, winter acclimatization, or among populations (Collins 1973; Stewart 1979; E. J. Glanville, pers. obs.). Therefore, both the warm and cold acclimation temperatures were below the thermal neutral zone. Using an equation for the oxygen consumption of bush rats at T_a s below the thermal neutral zone (Stewart 1979), and assuming no difference in T_b or conductance, a difference of 0.62 ml O_2 g^{-1} h^{-1} was found in resting metabolic rate of a bush rat between 12°C and 22°C. Consequently, a 115-g bush rat at rest needs 33.8 kJ extra energy per day when T_a is 12°C (using 19.77 J/ml O_2). However, we saw no significant increase in food consumption in CS rats and no change in body mass over the duration of cold acclimation. To estimate the energy savings conferred by the increased fur thickness and decreased T_b of cold-acclimated rats, we used a heat generation (Q_{gen}) model designed for an ellipsoid endotherm (Porter and Kearney 2009). We input the T_b (Table 1) and empirically determined values for mean fur thickness (Table 1) of rats in each treatment group and the median body mass (115 g) into the equation spreadsheet provided as supplementary material to Porter and Kearney (2009) to calculate the Q_{gen} of cold- and warm-acclimated bush rats at 12°C and 22°C. By comparing the Q_{gen} of cold-acclimated rats at 12°C to that of warm-acclimated rats at 12°C, the energy saving conferred by the reduced T_b and increased fur thickness of CE and CS rats was determined. The lower T_b and thicker fur of CE bush rats confers a daily saving of 14.6 kJ, 11% of daily food intake, compared to WE bush rats. The 0.6°C reduction in mean T_b accounted for 19.7% of the energy savings. The thicker fur of CS bush rats increased the daily energy saving to 15.6 kJ or 22% of daily food intake, of which 18.5% was due to the decrease in T_b . Therefore, the energy savings provided by the increased fur thickness and decreased body temperature (15.6 kJ/day in CS rats and 14.6 kJ/day in CE rats) account for approximately 45% of the estimated cold-induced increase in energy expenditure from the resting metabolic rate calculations (33.8 kJ/day).

Maintaining the T_b of a warm-acclimated rat in the cold requires increased energy expenditure, which necessitates increased food consumption if body mass is to be maintained. However, CS bush rats did not increase food intake and lost no weight over the time course of acclimation. Because body mass did not change, we assumed that the bush rats were in a state of energy equilibrium, and therefore we used food consumption as a surrogate for daily energy expenditure (Rezende et al. 2009). We assumed that digestibility did not change between acclimation treatments, because most studies suggest that thermal acclimation and season affect parameters such as the quantity of food consumed and the capacity of the gut rather than digestibility (Li and Wang 2005; Rezende et al. 2009; Zhao et al. 1995). Under these assumptions we found no difference in the estimated daily energy expenditure of CS and WS bush rats. This suggests that the thicker fur and lower T_b of CS bush rats compensated for any T_a -driven need to increase heat production and thus food consumption. Howev-

er, exercised rats did increase food consumption. Compared to sedentary rats, food intake increased by 50% in WE and by 81% in CE bush rats. Thus, the cold-induced increase in fur thickness and decrease in T_b of CE bush rats did not compensate completely for the increased energy and heat production demands of their environment. Low levels of enforced exercise in the cold approximately doubled food intake to maintain body mass. Our modeling suggests that without the increased fur thickness and decreased T_b CE rats would have had to increase food intake more substantially (by a further 14.6 kJ/day) to maintain condition. Increasing food intake in response to the cold is possible in captive animals, as they have an ad libitum food supply. However, in the natural environment lower T_{as} are concurrent with reduced food availability (Jackson et al. 2001; McDevitt and Speakman 1994; Porter et al. 2000; Scantlebury et al. 2005). Strategies such as increased insulation and decreased T_b reduce food requirements of cold-exposed mammals, reducing the need to increase foraging time and exposure to low T_{as} .

Cold-acclimated sedentary (CS) bush rats had significantly thicker fur than CE bush rats. The more dense fur of the sedentary rats can be explained by a greater need to increase insulation because maximum T_b is lower due to the absence of daily exercise. Spikes in T_b did correspond directly to exercise events in rats from the exercise treatment. Lower daily T_b maxima can result in increased fur thickness. Alternatively, increased fur thickness reduces the capacity of an endotherm to lose heat, which is required during intense exercise to prevent overheating. Therefore, fur thickness might not increase as much in response to cold in exercised animals compared to sedentary animals because of a greater need to lose heat. It also is possible that increased fur thickness decreases the efficiency of movement, so that a larger increase in fur thickness might impede mobility in small mammals (Boyles and Bakken 2007). To increase the energy savings afforded by the increased fur thickness and decreased T_b , cold-acclimated rats could also build nests with greater insulation. Although we did not quantify it, nest size was observed when enclosures were cleaned. The nest size of cold-acclimated bush rats did not appear to differ from that of warm-acclimated rats, nor was any difference in the nest size of exercised or sedentary rats apparent.

In conclusion, balancing the activity-induced increase in T_b with lower T_b minima allows exercised rats enduring cold environments to benefit from both maintained activity and any energy savings afforded by lower T_b s. Cold-induced lower T_b s are evident in a controlled environment with ad libitum food and persist even when exercise is enforced. We demonstrate that these reductions in T_b combine with increased fur thickness to decrease substantially the energy requirements of bush rats exposed to cold T_{as} .

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